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DOI: https://doi.org/10.1016/j.rse.2008.10.019

Editorial Manager(tm) for Remote Sensing of Environment
Manuscript Draft

Manuscript Number: RSE-D-07-00625R2

Title: Retrieval of Foliar Information about Plant Pigment Systems from High Resolution Spectroscopy

Article Type: Imaging Spectroscopy Special Issue

Keywords: plant pigments; chlorophyll a, b; carotenes; xanthophyll pigments; anthocyanin pigments; absorption features; spectral measures of pigments; spectroscopy

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Retrieval of Foliar Information about Plant Pigment Systems from High Resolution Spectroscopy

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Abstract

Life on Earth depends on photosynthesis. Photosynthetic systems evolved early in Earth history and have been stable for 2.5 billion years, providing prima facie evidence for the significance of pigments in plant functions. Photosynthetic pigments fill multiple roles from increasing the range of energy captured for photosynthesis to protective functions. Given the importance of pigments to leaf functioning, greater effort is needed to determine whether individual pigments can be identified and quantified *in vivo* using high fidelity spectroscopy. We review recent advances in detecting plant pigments at the leaf level and discuss successes and reasons why challenges remain for robust remote observation and quantification. New methods to identify and quantify individual pigments in the presence of overlapping absorption features would provide a major advance in understanding their biological functions, quantifying net carbon exchange, and identifying plant stresses.

Introduction

Life on Earth is driven by photosynthesis, producing both oxygen and organic matter (Nelson & Yocum, 2006). Photosynthesis is one of the earliest biological processes to evolve and the pigment systems in modern photosynthetic bacteria, algae, and higher plants appeared at least 2.5 billion years ago (Olson & Blankenship, 2004; Kiang et al., 2007). Photosynthetic pigments including chlorophylls a, b, and several carotenoid pigments, date from this period. The length and stability of this record, and the various roles of pigments in photosynthesis, protection and defense, demonstrate the functional importance of their composition, associated protein complexes, and chloroplast structure. Their significance for life on Earth provides the rationale for improving our capability to remotely measure them. Furthermore, the stability of chlorophyll molecules makes them a target, along with water, in the search for extraterrestrial life (Arnold et al., 2002; Wolstencroft & Raven, 2002; Seager et al., 2005). The range of characteristic reflectance patterns varies among different taxonomic groups of photosynthetic organisms in the visible and near-infrared spectrum as illustrated in Figure 1 (Kiang et al., 2007). Although terrestrial and aquatic plants and mosses share a common photosynthetic apparatus, in contrast to some algae, lichens, and bacteria, reflectance differences among different taxa exist across the range of wavelengths that relate to both structure and biochemistry.

Insert Figure 1 about here

Developing methods to quantify pigment content (grams pigment/ leaf area), concentration (grams pigment/gram dry weight) and pigment composition from remotely sensed data would clearly provide a capability that could advance understanding of photosynthetic processes (e.g., light regulation, photooxidation, chlorophyll fluorescence) and provide insight into detection and monitoring of foliar condition (e.g., environmental stressors). This review provides a brief summary of the major plant pigments and their ecophysiological functions and the most widely used spectroscopic methods for retrieving information from high spectral resolution remote sensing. This paper is dedicated to Professor Alexander F.H. Goetz, who led a major investigation into the potential for measuring plant biochemistry from narrow-band imaging spectrometry in the early 1990s. This activity stimulated international research in the detection of plant biochemicals and many advances in measuring leaf pigments from reflectance data originate from this effort. To narrow the scope of this review, we focus primarily on reflectance measurements, at the leaf level, emphasizing advances in the past 15-20 years, and examining two types of quantitative approaches: (1) empirical and semi-analytical methods and (2) physically based radiative transfer models and quantitative methods.

Photosynthesis and Other Functions of Leaf Pigments

Absorption of light in the visible spectrum by plant pigments produces a unique spectral reflectance signature. Light is captured by the process of photosynthesis (Govindjee & Krogmann, 2004) and the light energy is stored as carbohydrate, through a series of

electron transfers that occur on the thylakoid membranes in chloroplasts. In the intact chloroplast, pigment-protein complexes are organized into two photosystems that harvest light and transfer energy to the reaction centers. Besides chlorophyll a and b, the photosynthetic antenna (the organized association of pigments that capture photons and transfer energy to the reaction centers) contain other membrane-bound accessory pigments that include β-carotene, lutein and xanthophyll cycle pigments (Lichtenthaler, 1987). Figure 2 shows the absorption spectra of chlorophyll a and b and β-carotene. Accessory pigments increase the spectrum over which light can be absorbed and also perform other functions, not all of which are fully understood, but which alter the efficiency of photon capture and/or provide protective and defensive functions, e.g., avoiding damage to the reaction centers under excess UV light or freezing temperatures. For example, under high illumination conditions, light intensity may exceed the capacity for electron transfer between the photosystems and some carotenoid pigments, such as xanthophylls, are involved with protection from photooxidation and photoinhibition (Demmig-Adams & Adams, 1996) and with release of excess solar energy through induced leaf fluorescence.

Insert Figure 2 about here

Measuring the Absorption Spectra of Foliar Pigments from high resolution spectroscopy

95 One factor that has limited our ability to quantify individual pigments from reflectance
96 data is that we do not precisely know their absorption characteristics. It has long been
97 noted (Rühle & Wild, 1979) that extracted chlorophyll absorption peaks are shifted about
98 20 nm to shorter wavelengths than observed in reflectance from intact leaves. The
99 wavelength positions of maximum absorption vary with the extraction solvent used, due
100 to differences in polarity, and the loss of pigment-protein interactions (Porra, 2002). The
101 absorption maxima of chlorophyll a when extracted in diethylether are at 430 and 662
102 nm, and chlorophyll b has peaks located at 453 and 642 nm (Figure 2). β-carotene
103 extracted in hexane absorbs at 451 and 470 nm (Du et al., 1998). In the presence of
104 multiple accessory pigments and non-photosynthetic cytoplasmic pigments like
105 anthocyanin, it becomes clear why identification and quantification of individual
106 pigments in the intact leaf has been difficult to obtain from their spectral absorptance
107 signatures at specific bands (Buschmann et al., 1994). Multiple scattering from within
108 the leaf further complicates pigment retrievals making remote detection even more
109 challenging, as further discussed below.
110
111 A large number of narrow-band spectral methods have been proposed to detect plant
112 pigments, ranging from simple band ratios to radiative transfer methods. Indexes
113 currently used for estimating chlorophyll from leaf optical properties exploit the
114 differences in reflectance between healthy and stressed vegetation in the visible and the
115 red edge (Horler et al., 1983; Vogelmann et al., 1993; Carter, 1994; Carter & Spiering,
116 2002; Zarco-Tejada et al, 2001; Sims & Gamon; 2002, Malenovský et al., 2006). These
117 indexes are classified into red / NIR ratios, green and red edge indexes, and derivative

118 indexes. Table 1 provides examples of these indexes; see more comprehensive reviews
119 of chlorophyll indexes in Blackburn (1998, 2007), le Maire (2004) and Zarco-Tejada et
120 al. (2005a, 2005b).
121
122 Insert Table 1 about here.
123
124 Grossman et al. (1996) tested various regression methods on several leaf datasets and
125 concluded that maximum correlations were produced at different wavelengths, which
126 depended on the index and whether the pigment was expressed on an area or mass basis.
127 Blackburn (1998) reviewed the ability of several ratio-based indexes, $RARS_{a, b}$ $PSSR_{a, b}$
128 and $PSND_{a, b}$ (Table 1) and first and second derivatives of the reflectance and the log 1/R
129 (Yoder and Pettigrew-Crosby, 1995) to predict contents of chlorophyll a, b and
130 carotenoids, and found best performance from PSSR and PSND indexes. Blackburn
131 (1998, 2007) found strong but non-linear relationships, typically either power or
132 exponential fits, could be developed at either leaf or canopy scales. He concluded that
133 maximum correlations varied with wavelengths, index, and whether the analysis was on
134 an area or mass basis.
135
136 Despite problems in identifying specific wavebands for individual pigments, the earliest
137 attempts to use spectroscopy focused on estimating the content of total photosynthetic
138 pigments using ratios of different spectral bands (e.g., simple ratio as R_{NIR}/R_{red}) or the
139 normalized difference vegetation indexes (Table 1). As interest in narrow-band
140 spectrometry increased, other ratio-based indexes or simple transforms of band

combinations were developed as practical methods of analysis. Similarly, the use of first and second derivatives, which were applied across the full spectrum, came into common use in the 1990s. Derivative analyses, discussed in the following section, are primarily focused on the red edge, which is the long wavelength edge of the chlorophyll absorption, in the wavelengths between 700-750nm.

From the beginning of systematic earth observation, remote sensing has focused on measuring plant "greenness", often described as synonymous with chlorophyll content. There is a close relationship between photosynthetic capacity, estimated by pigment contents, and net primary production that is captured to first approximation by the greenness indicators measured by broad-band multispectral instruments (Gates et al., 1965; Monteith, 1976; Sellers, 1985, 1987; Asrar et al., 1984). Many band combinations in the visible spectrum have been used to estimate total pigments as illustrated by the examples shown in Table 1. This list is not exhaustive but it does cover the commonly used methods for leaf analysis, while also including more recent examples.

The empirical nature of the early studies, although based on a physiological understanding of absorption spectra of photosynthetic pigments, produced variable results when applied to new conditions due to the range of analytical methods used, experimental conditions, and characteristics of the species. At the leaf scale, when applied to a limited number of species or phenologic conditions, such as in agricultural fields or grasslands, indexes have provided good results despite lack of agreement on

optimal methods, as attested to by the extensive literature. Despite the search for a universal method, no one method has been adopted as satisfactory under all growth and environmental conditions.

Measuring Photosynthetic Capacity in Chlorotic and Healthy Leaves

Within a species, a variety of factors, including growth stage, irradiance, and various environmental stress conditions, can change the total pigment content and the chlorophyll a:b ratio (Anderson et al., 1988). Changes in the wavelength of the red edge inflection point position (IPP) have been observed for different species (Kiang et al., 2007), time during the growing season (Gates et al., 1965; Horler et al., 1983; Belanger, 1990) and from environmental stresses (Chang & Collins, 1983; Milton et al., 1983; Ustin and Curtiss, 1990; Hoque and Hutzler, 1992). Chlorosis increases reflectance across the visible spectrum and causes a shift to shorter wavelengths (blue-shift) of the red-edge IPP, due to narrowing and a reduction magnitude of the chlorophyll absorption feature and a reduction in depth (Ustin and Curtiss, 1990). Gates et al. (1965) and Collins (1978) provided early observations of a blue shift of the red edge which was attributed to the loss of chlorophyll. Rock et al. (1988) noted a disproportional loss of chlorophyll b accompanied by a blue shift of the IPP for foliage exposed to air pollution. In contrast, under increased chlorophyll content, the chlorophyll absorption feature deepens and broadens (e.g., Buschmann and Nagel, 1993) causing a red-shift of IPP (Collins et al., 1978). Kiang et al. (2007) note that the red-edge shifts of IPP from shorter to longer wavelengths for species ranging from algae, lichens, mosses, aquatic and terrestrial

187 plants. Thus, this index is best applied to variation within a vegetation type to detect
188 stress and between types when it is used to identify taxa. Several index methods have
189 been used to detect red-edge shifts in narrow band spectra. (Table 1).
190
191 Insert Figure 3. about here
192
193 **Using the Red edge to Detect Stress**
194
195 Most early studies of the red edge used a first or second derivative or the amplitude of the
196 derivative to identify the wavelength at the inflection point, which was then correlated
197 with chlorophyll content (Horler et al., 1983; Wessman, 1990). Figure 3 illustrates how
198 environmental stressors, in this case oil contamination, in a grassland site with relatively
199 little variation in leaf area index can affect the wavelength position of the inflection point
200 for chlorophyll absorption (Figure 3a) and how the IPP can be used to map spatial
201 variation in chlorophyll content due to the oil contamination (Figure 3b).
202 Despite 30 years of research, defining the relationships between pigment content and the
203 red edge using various spectral indexes, the red edge remains an area of active research
204 (e.g., Gitelson et al., 1996a; le Maire et al., 2004; Asner et al., 2005; Zarco-Tejada et al.,
205 2005a; Sims et al., 2006). Although the use of derivatives to estimate the red edge is
206 widely used, because of their sensitivity to noise, other methods e.g., the inverted
207 Gaussian of Miller et al. (1990) are now preferred. Recently, Cho and Skidmore (2006)
208 proposed an improved method to estimate the red-edge inflection wavelength using
209 intersecting lines originating from the shoulders of the derivative. More research is

210 needed to understand precisely what controls the position of long wavelength side of the
211 chlorophyll absorption band. For example, Curtiss & Ustin (1989) observed a
212 broadening of the chlorophyll absorption band in ponderosa pine needles following
213 exposure to atmospheric ozone, which they interpreted as a red-shift that mimicked
214 increased chlorophyll content rather than a blue-shift which is expected under lower
215 chlorophyll content. This effect was hypothesized to be due to increasing disorder in the
216 chloroplast with ozone exposure, a pattern consistent with observations that an early sign
217 of ozone injury is granulation of the thylakoid membranes. Recently Noomen et al.
218 (2006) reported that exposure to natural ethane gas caused a small red shift in corn
219 reflectance and a significant decrease in absorption in the 550-750nm region.
220
221 **Leaf Fluorescence and Stress Detection**
222
223 Use of remotely sensed fluorescence to estimate photosynthetic activity began in the
224 oceanography community to detect phytoplankton productivity (e.g., Kim, 1973). For
225 both marine and terrestrial applications, two types of systems have been used, active and
226 passive lasers. Marine systems usually use lasers in the blue spectral region to excite
227 photosystem II activity, with chlorophyll fluorescence being observed in the 730 nm
228 region. Passive systems rely on measuring natural fluorescence stimulated by sunlight.
229 Although the signal is small, as spectral resolution in the visible spectrum and
230 signal/noise in spectrometers have increased, interest in using this technology to directly
231 observe photosynthetic functioning has become of more interest to plant physiologists
232 and ecologists (Lichtenthaler, 1988). Figure 4 shows the wavelength specific absorption

233 of light excitation by a UV laser and corresponding chlorophyll fluorescence emission for
234 a typical leaf.
235
236 Several authors have noted a double peak in first derivative of leaf reflectance around
237 700-725 nm (Horler et al., 1983; Boochs et al., 1990). It was later shown that the
238 reflectance derivative could exhibit several local maxima in the red edge area in
239 measured (Gitelson et al., 1996a) and simulated (le Maire et al., 2004) spectra. The
240 nature of this multiple-peak feature is not well understood. Zarco-Tejeda et al. (2003)
241 suggested that double-peak is due to steady-state fluorescence emission. Le Maire et al.
242 (2004) showed that the double-peak can be simulated by the PROSPECT model by
243 increasing the chlorophyll content alone, regardless of the values of other parameters. It
244 can be also simulated by increasing the structure parameter alone if the total chlorophyll
245 content exceeds 400 mg cm^{-2}.
246
247 The potential to use fluorescence spectroscopy as a non-destructive method to detect
248 plant stress was recognized several early studies (e.g., Buschmann & Schrey, 1981;
249 Chappelle et al., 1984; Lichtenthaler & Rinderle, 1988). Fluorescence emission maxima
250 were observed at 440-450, 525-535, 680-685, and 735-740 nm (e.g., Richards et al.,
251 2003) and related to components of photosynthesis and leaf pigments (e.g., Buschmann et
252 al., 2000). Gitelson et al. (1999) used a ratio to estimate fluorescence at 735/700nm
253 which provided a near linear prediction of chlorophyll content in intact leaves. Recent
254 authors have continued to explore active (e.g., laser-induced) fluorescence (Corp et al.,
255 2006; Richards et al., 2003) and solar-induced fluorescence (Zarco-Tejada et al., 2003;

Dobrowski et al., 2005) to detect environmental stresses. Carter et al. (2004) measured solar-induced fluorescence in 10 nm bands at 690 and 760 nm using the Fraunhofer Line Depth Principle to detect stress, and more recently, Merconi and Colombo (2006) used very high resolution (0.06 nm) spectrometry at 687 and 760 nm to detect chlorophyll fluorescence in an oxygen band where solar irriadiance is reduced.

Insert Figure 4 about here

Detecting and Quantifying Foliar Pigment Composition in Relation to Ecological Condition

The concept of ecological convergence expresses the "economy of form" or the efficiency that particular sets of traits provide in exploiting limited environmental resources (Field et al., 1992). This concept implies that the cost of biochemical investments should be related to the growth potential and thus better measurements of pigment distributions and concentrations could provide a basis for monitoring physiological and ecological processes. It is known that pigment composition varies with species and environmental conditions (e.g., Peñuelas et al, 1995a; Carter and Knapp, 2001; Asner et al., 2008). Deciduous species have leaves that are generally adapted for faster growth rates and higher photosynthetic capacity (e.g., higher chlorophyll and nitrogen concentrations) than needles in evergreen species where the cost of producing lower photosynthetic capacity foliage can be amortized over several years (Wright et al., 2004). Figure 5 illustrates differences in amount and composition of photosynthetic pigments in leaves from a deciduous oak grown in the higher light environment of the

279 savanna compared to leaves from the more closed canopy of the evergreen oak
280 community (Ustin et al., 1993). Reflectance from the evergreen oak leaves is lower
281 across the visible spectrum and the spectral shape is different. The total amount of
282 pigments is lower in leaves from the evergreen oak and their relative proportions differs
283 from the deciduous species. Note that small absorption features (at this presentation
284 scale) are observed in these leaves near 585 nm, 620 nm, and 650 nm, suggesting that it
285 may be possible to identify the basis for these features, particularly at the canopy level
286 where absorption features are enhanced by transmission through multiple leaf layers
287 (Allen and Richardson, 1968; Knipling, 1970; Curran, 1980; Stylinski et al., 2001;
288 Roberts et al., 2004).
289
290 Insert Figure 5 about here
291
292 **Detection of foliar stress using the Photochemical Reflectance Index (PRI)**
293
294 Under high light, it is well established that xanthophyll cycle pigments function to
295 prevent oxidation of the reaction centers (Demmig-Adams & Adams, 1996). There is
296 also strong evidence for optimization of photon capture efficiency at low light (Gamon et
297 al., 1990; Horton et al., 1994; Bailey et al., 2001). Short-term changes in reflectance in
298 response to the light environment are observed near 530 nm that detect reversible
299 changes in the distribution of xanthophyll cycle pigments (violaxanthin is converted to
300 zeaxanthin through the intermediate antheraxanthin under high light and reverts to
301 violaxanthin under low light; Demmig-Adams, 1990). This is the basis for the

302 photochemical reflectance index (PRI; Table 2), a normalized ratio of 531 nm to 570 nm
303 developed by Gamon and colleagues (Gamon et al., 1990, 1992, 1993, 1997; Peñuelas et
304 al., 1995a, 1997). The PRI, developed by Gamon at the time of the NASA Accelerated
305 Canopy Chemistry Program (ACCP), has been shown to detect the transition to
306 violaxanthin in foliage exposed to high light intensities and this response is closely tied to
307 photosynthetic activity (Gamon et al., 1990, 1992, 1993; Peñuelas et al., 1995a).
308 Conversely, leaves grown under low light have low zeaxanthin and little PRI response
309 (Peñuelas et al., 1995b). These relationships have been verified at the leaf level in
310 multiple studies in the years since this index was developed (e.g., Sims and Gamon 2002;
311 Nakaji et al., 2005; Nichol et al., 2000). When the PRI is measured over longer time
312 spans (seasons, years) or across species, variation appears to track relative composition of
313 chlorophylls and carotenoids (e.g. Nichol et al., 2000; Rahman et al., 2001, Rahman and
314 Gamon, 2004; Asner et al., 2005, 2006; Drolet et al., 2005; Fuentes et al. 2006).
315
316 **Insert Table 2 about here**
317
318 **Detection of Carotenoid Pigments and Anthocyanin Pigments**
319
320 The red and yellow colors of autumn foliage are seen because of the change in
321 photoperiod (short days/long nights) and/or low temperatures that initiate a senescence
322 response in which chlorophyll pigments breakdown before the carotenoid pigments (e.g.,
323 lutein and β-carotene). Table 2 lists several wavelength specific indexes that have been
324 used to estimate carotenoid pigment contents in foliage. Anthocyanins are red flavonoid

pigments that are cytoplasmic and not associated with the chloroplast but they are often observed during environmental stresses (e.g., low or high temperatures) and during senescence (Schaeberg et al., 2008). Anthocyanins are also common during the earliest stages leaf development before the photosystems are fully functional (Gamon & Surfus, 1999). Like carotenoids, anthocyanins protect the photosynthetic system from excess light, particularly excess UV radiation (Merzlyak & Chivkunova, 2000; Gitelson et al., 2001), and may provide other functions, e.g., protection from herbivory and fungal pathogens (Close & Beadle, 2003). These pigments have a single absorption maximum around 529 nm and can be detected by reflectance changes in the green region (Table 3); reflectance in the red-edge region does not vary with anthocyanin content and so the red edge can be used as a reference against which anthocyanin is determined (Curran et al., 1991; Neill & Gould, 1999; Gitelson et al., 2001, 2006).

Insert Table 3 about here.

Development of Spectral Vegetation Indexes for Individual Pigments

Detection of individual pigments from reflectance data has been given less attention by the remote sensing community than total pigments, despite the importance of accessory pigments in light capture, photosystem protection, and in various growth and development functions. This lack of research stems from the difficulty in resolving the overlapping absorptions of individual pigments and their high degree of correlation (Chappelle et al., 1992; Ramsey & Rangoonwala, 1995; Blackburn, 1998, Grossman et

348 al. 1998). Nonetheless, because pigments have distinctive absorption spectra depending
349 on their molecular structure and local chemical environment, there is a potential to
350 measure these properties using reflectance spectroscopy. The challenge is to properly
351 account for the multiple factors influencing the retrieved signal. While many models
352 relate chlorophyll content to reflectance (e.g., Gitelson & Merzlyak, 1994, Sims &
353 Gamon, 2002; Richardson et al., 2002; Gitelson et al., 2003; le Maire et al., 2004) and
354 some are robust in chlorophyll prediction, only few models support retrieval of
355 anthocyanins and carotenoids (e.g., Gitelson et al., 2001, 2002; Sims & Gamon, 2002).
356
357 There are a few examples in the literature where chlorophyll a and chlorophyll b have
358 been separately assessed from reflectance data using empirical models. Among the first,
359 Chappelle et al. (1992) and Blackburn (1999) used band ratios to quantify chlorophyll b.
360 Curran et al. (2001) demonstrated retrieval of foliar chlorophyll b using the continuum
361 removal method of Kokaly & Clark (1999). Pinzón et al. (1998) used a hierarchical
362 singular value decomposition method to quantify biochemical constituents in intact leaf
363 samples, where total sample variance was broken down into smaller ranges of variation
364 using a series of weighting vectors.
365
366 One explanation for past inconsistencies in separating and quantifying different pigments
367 is because their absorption spectra overlap, thus simple methods do not account for the
368 interacting effects of multiple pigments and fail across a range of taxa (e.g., Figure 1) or
369 where the conditions are outside the range that these empirical methods were tested and
370 calibrated. In other cases, indexes that seem to provide consistent results at the leaf level,

fail or become inconsistent at the canopy or stand levels, in part due to the complexities of the three-dimensional structure and multiple scattering in intact leaves. Additionally, despite progress at the leaf level, no analytical model today has accounted for the quantification of anthocyanin and carotenoid contents at the canopy or stand scales.

Development of Multiple Pigment Models

The first model to estimate content of multiple pigments was developed by Gitelson and colleagues (Gitelson et al., 2003, 2006). They presented the analytical development and underlying hypothesis for a three-band model for estimating pigment content in plant leaves. This conceptual semi-analytical model is based on the relationship between the reciprocal of reflectance, a property that is closely related to the infinite reflectance of a leaf, and the inherent optical properties, absorption and backscattering coefficients. With this semi-analytical approach, they modeled pigment absorption using three narrow spectral bands, where reflectance in the first band (λ_1) is maximally sensitive to absorption by the pigment of interest but is also affected by absorptions from other pigments and variability in backscattering (Figure 6). These extraneous effects are removed by identifying a second band λ_2 where the other pigments absorb but the pigment of interest has little effect and where backscattering is relatively unchanged from λ_1. Then a third spectral band λ_3 is selected where backscattering controls the overall reflectance. Combining these three bands allows the pigment content ($C_{pigment}$) to be estimated:

$$C_{pigment} \propto [R^{-1}(\lambda_1)-R^{-1}(\lambda_2)]\times R(\lambda_3).$$

392 The spectral regions used in the model were tuned with respect to the pigment of interest
393 and the optical characteristics of the leaves studied. The optimal bands for each pigment
394 (chlorophyll, carotenoids and anthocyanins) retrieval are determined by performing the
395 calibration for a continuous range from 400-800nm, isolating one band at a time, and
396 choosing each of the 3 bands according to a minimal root mean square error (RMSE)
397 (Figure 6). For total chlorophyll content retrieval optimal λ_1 was found in either the green
398 (540-560 nm) or red edge (700-730 nm) range, and $\lambda_2 = \lambda_3$ was in the NIR beyond 760
399 nm (Figure 6a, Gitelson et al., 2003, 2006).
400
401 Insert Figure 6 about here.
402
403 Zur et al. (2000) and Gitelson et al. (2002) identified a spectral band near 510 nm that was
404 sensitive to total content of carotenoids and used it in a three-band model to estimate the
405 total carotenoid content (Figure 6b, Gitelson et al., 2002, 2006). They applied this model
406 to retrieve anthocyanin, developing an Anthocyanin Reflectance Index (Figure 6c,
407 Gitelson et al., 2001; 2006). In anthocyanin-free leaves, both the green and the red edge
408 bands can be used as λ_1 for chlorophyll estimation and as λ_2 for carotenoid estimation
409 (Figure 6b). Thus, only four spectral bands are required to retrieve three pigment
410 contents: 510–520 nm (carotenoids), 540–560 nm (anthocyanins), 700–730 nm (total
411 chlorophyll) and NIR in the range 760–800 nm. This model produced accurate estimates
412 of the total chlorophylls, carotenoids, and anthocyanin contents, explaining more than
413 91%, 70%, and 93% of the variance, respectively (Gitelson et al., 2006). However,
414 models for carotenoids and anthocyanin retrieval was found to be species specific.

Leaf Radiative Transfer Models

Physically based RT models have the potential to produce more accurate and consistent predictions of pigment interactions because they are based in physics and use the full spectrum rather than individual bands and therefore, do not require calibration each time they are used. However, RT model predictions of the optical properties of monocot or dicot leaves or needles depends on how well understood all processes affecting reflectance are and how they are accounted for in the models. Although they have potential to predict pigment content more consistently and accurately then empirical methods, they require more input parameters, which if wrong result in poor model performance. Thus, empirical models can be more accurate than physical models if the components are improperly modeled or the input data is wrong.

Several leaf models, e.g., PROSPECT (*Leaf Optical Properties Spectra*, Jacquemoud & Baret, 1990), LIBERTY (*Leaf Incorporating Biochemistry Exhibiting Reflectance and Transmittance Yields*, Dawson et al., 1998), LEAFMOD (*Leaf Experimental Absorptivity Feasibility MODel*, Ganapol et al., 1998), and SLOP (*Stochastic model for Leaf Optical Properties*, Maier et al., 1999; Maier, 2000), have been used since the 1990s to estimate total chlorophyll concentration (see review in Ustin et al., 2004). Instead of modeling multiple pigments, RT models currently assume that leaf pigments are entirely composed of chlorophyll and that the horizontal and vertical distribution of these absorbers is homogeneous within foliar tissues.

438 Characterization of *in situ* absorption coefficients for xanthophylls, carotenes and even
439 chlorophyll b are needed to be added to leaf RT models to estimate individual pigments.
440 This will require defining *in vivo* absorption coefficients for all individual pigments but
441 also better knowledge of the spectral variation of the refractive index of leaves,
442 information that is not currently available, and which may vary depending upon the
443 chemical environment within the leaf. Maier et al. (1999) and Berdnik and
444 Mukhamed'yarov (2001) followed this approach and utilized separate *in vitro* absorption
445 spectra of chlorophyll a, b and several carotenoids in developing their leaf optical
446 properties model. An advanced version of the PROSPECT model (PROSPECT 5) that
447 can discriminate chlorophylls from total carotenoids has been developed (Feret et al.,
448 2008). This required a long phase of calibration using datasets carefully selected to cover
449 a wide range of leaf photosynthetic pigments. Feret et al. (2008) showed that some
450 improvements in prediction of leaf reflectance, on the order of 5% in the visible, could be
451 made by better characterization of pigment contributions. Figure 7 shows the comparison
452 between measured and PROSPECT-5 predicted total chlorophyll (left) and total
453 carotenoid (right) contents from leaves representing a wide range of ecological conditions
454 and communities, from mesic and semiarid temperate forests, shrublands, and agriculture,
455 and from subtropical and tropical systems.
456
457 Insert Figure 7 about here.
458
459 The fact that light can be propagated through leaves without encountering foliar
460 pigments, for instance in veins, is a phenomenon known as the sieve effect which has

been accounted for in RT models to better interpret absorption spectra of leaves (Latimer, 1983; McClendon & Fukshansky, 1990). Additionally, bifacial leaves with different chlorophyll content in the palisade and spongy mesophylls can be simulated using the Kubelka-Munk theory applied to two or more stacked layers (Yamada & Fujimura, 1991; Richter & Fukshansky, 1996). With this added level of physical detail being built into RT leaf reflectance models, it is likely that it will lead to better characterization of individual pigments.

A recent European program to develop methods to detect leaf and canopy fluorescence has produced a new model, FluorMOD, with linked leaf and canopy models to detect the effects of steady-state solar-induced chlorophyll fluorescence (Zarco-Tejada et al., 2006; Middleton et al., 2008).

Challenges in Imaging Spectroscopy of Vegetation Pigments

The application of narrow-band spectral methods for pigment detection to airborne and spaceborne spectrometers has been the intended goal of much of the leaf-level research since the time of the ACCP program that Dr. Goetz headed. Field- and laboratory-based spectroscopy of plant pigments has had a long period of development and today, a large number of studies have explored the detection of pigments in many leaf types originating from a wide range of ecosystems and using a wide range of methods. The use of airborne and space-based imaging spectrometers to detect and map foliar pigments is still relatively new (e.g., Collins, 1978; Rock et al., 1988; Zarco-Tejada & Miller, 1999). These examples highlight the potential contribution of imaging spectroscopy for

484 detecting and quantifying foliar pigments from imaging spectroscopy to studies of
485 canopy physiology and ecology. At this time, current methods have not delivered
486 unambiguous results and operational methods for this level of biochemical retrieval.
487 Advances in algorithm development as highlighted above indicate that numerous
488 challenges remain to be solved before pigment concentrations can be routinely retrieved
489 from space. Imaging spectrometer design is a key issue in the quest for more quantitative
490 approaches to identification of pigments. This will require an instrument that has high
491 spectral resolution, ~3-5nm to measure pigment details but which has good signal to
492 noise characteristics of up to 1000:1 in the visible and infrared region. The fidelity of the
493 sensor, which includes signal-to-noise performance, uniformity of the image, and
494 stability of the electronics all affect the outcome of a canopy chemical analysis.
495 Instruments must be calibrated in terms of wavelength (i.e., avoiding keystone and smile)
496 and have radiometric stability.
497 A good example of current technological capability is found by looking at the
498 evolution of the Jet Propulsion Laboratory's Advanced Visible Infared Imaging
499 Spectrometer (AVIRIS) program, which started under the leadership of Dr. A.F.H. Goetz
500 in 1982. In the early 1990s, AVIRIS signal-to-noise ratio was in the 10-100 range
501 depending upon wavelength region (Vane et al., 1993). Since then, AVIRIS has evolved,
502 through major upgrades and constant hardware and software adjustment, to provide
503 spectra with effective signal-to-noise performances of many 100s to 1000s (Green et al.,
504 2003). The resolution, stability, and sensitivity of the sensor technology bears squarely
505 upon the state of hyperspectral algorithm development. With the high fidelity of AVIRIS
506 today and a few other sensors, it is possible to use physically-based methods, such as

canopy RT models, to explore spectra in some detail. Thus, advances in modeling pigments at the leaf level can be incorporated into models suitable for imaging spectrometers. However, since most remote mapping of ecosystems will be at canopy level, solid coupling of leaf radiative transfer models with canopy scale models will be key to successful retrieval of multiple pigments from air- or spaceborne spectrometers. At canopy scale, the simultaneous assessment of canopy heterogeneity as well as pigments will require a better representation of clumped and sparse canopies in coupled RT models. Future sensors, having capabilities similar to the proposed NASA HyspIRI mission and the German EnMap program (Kaufmann et al., 2006), will eventually deliver spectroscopic measurements of sufficiently high fidelity to advance the mapping of canopy pigments and other chemicals.

Acknowledgements: This paper is dedicated to Dr. Alexander Goetz, who as chair of the NASA HIRIS Canopy Chemistry Program stimulated much of the early research on plant pigments and continues to impact research today, through the use of plant biochemical databases and various analytical approaches that developed during this program. His vision of imaging spectrometry has allowed the development of techniques and measurements that have linked a detailed understanding of plant physiological functioning with optical properties, and provided the opportunity to study biological processes from the leaf to planetary scales. S.U. wishes to thank the Department of Biological Science, Victoria University, Wellington, New Zealand for providing space and support while this paper was written during my sabbatic leave and support from the

530 USDA Agricultural Experiment Station Hatch program, #5661-H “Identification of
531 canopy chemistry using reflectance and imaging spectroscopy”.

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List of Tables

Table 1. Spectral indexes developed as chlorophyll indicators.

Index	Short	Formula	Source
Single wavelengths			
Reciprocal Reflectance	**RR**	R_{700}^{-1}	Gitelson et al. (1999)
Logarithm of Reciprocal Reflectance	**LRR**	$\log R_{737}^{-1}$	Yoder & Pettigrew-Crosby (1995)
Combinations of wavelengths			
Waveband Ratios	**Ratios**	R_{NIR}/R_B , R_{NIR}/R_G , R_{NIR}/R_R , R_{NIR}/R_{RE}, R_B/R_G , R_B/R_R , R_R/R_G	Aoki et al. (1980, 1986), Vogelmann et al. (1993), Carter (1994), Gitelson & Merzlyak (1994, 1996, 1997), Datt (1998), Maccioni et al. (2001), Sims & Gamon (2002)
Modified Red-edge Ratio	**mSR**	$mSR = (R_{750} - R_{445})/(R_{705} - R_{445})$	Sims & Gamon (2002)
Pigment Specific Simple Ratio	**PSSR**	$PSSR_a = R_{800}/R_{675}$ $PSSR_b = R_{800}/R_{650}$	Blackburn (1998, 1999), Sims & Gamon (2002)
Ratio Analysis of Reflectance Spectra	**RARS**	$RARS_a = R_{675}/R_{700}$ $RARS_b = R_{675}/(R_{650} \times R_{700})$	Chappelle et al. (1992), Blackburn (1999)
Normalized Difference Vegetation Index	**NDVI**	$NDVI = (R_{NIR} - R_R)/(R_{NIR} + R_R)$	Datt (1998)
Red-edge NDVI	**mNDVI**	$mNDVI = (R_{750} - R_{705})/(R_{750} + R_{705})$	Gitelson & Merzlyak (1994), Gamon & Surfus (1999), Datt (1999), Sims & Gamon (2002))
Modified Red-edge Normalized Difference Vegetation Index	**mNDI**	$mNDI = (R_{750} - R_{705})/(R_{750} + R_{705} - 2R_{445})$	Sims & Gamon (2002)
Green NDVI	**gNDVI**	$gNDVI = (R_{750} - R_G)/(R_{750} + R_G)$	Gitelson et al. (1996), Datt (1998, 1999)
Pigment Specific Normalized Difference	**PSND**	$PSND_a = (R_{800} - R_{675})/(R_{800} + R_{675})$ $PSND_b = (R_{800} - R_{650})/(R_{800} + R_{650})$	Blackburn (1998)
Eucalyptus Pigment Indexes	**EPI**	$Chl_{a,b} = \alpha_{a,b} \times (R_{672}/(R_{550} \times R_{708}))^{\beta_{a,b}}$	Datt (1998)
Summed Reflectance Index	**SRI**	$S_1 = \int_{700}^{750} (R_\lambda/R_{555} - 1) d\lambda$ $S_2 = \int_{700}^{750} (R_\lambda/R_{705} - 1) d\lambda$	Gitelson & Merzlyak (1994)
Red edge position			
Red Edge Inflexion Point Position	**IPP**	$R''(\lambda_i) = 0$	Horler et al. (1983), Curran et al. (1995), Lichtenthaler et al. (1996), Kochubey & Kazantsev (2007)

Table 2. Spectral indexes developed as carotenoid indicators.

Index	Short	Formula	Source
Ratio Analysis of Reflectance Spectra	**RARS**	$RARS_c = R_{760}/R_{500}$	Chappelle et al. (1992)
Structure Insensitive Pigment Index	**SIPI**	$PSND_c = \left(R_{800} - R_{445}\right)/\left(R_{800} - R_{680}\right)$	Peñuelas et al. (1995a), Sims & Gamon (2002)
Pigment Specific Simple Ratio	**PSSR**	$PSSR_c = R_{800}/R_{500}$	Blackburn (1998)
Carotenoid Reflectance Index	**CRI**	$CRI_{550} = R_{510}^{-1} - R_{550}^{-1}$ $CRI_{700} = R_{510}^{-1} - R_{700}^{-1}$	Gitelson et al. (2002)
Modified Carotenoid Reflectance Index	**mCRI**	$mCRI_G = \left(R_{510-520}^{-1} - R_{560-570}^{-1}\right) \times R_{NIR}$ $mCRI_{RE} = \left(R_{510-520}^{-1} - R_{690-710}^{-1}\right) \times R_{NIR}$	Gitelson et al. (2006)
Photosynthetic Reflectance Index	**PRI**	$PRI = \left(R_{531} - R_{570}\right)/\left(R_{531} + R_{570}\right)$	Gamon et al (1990)
Eucalyptus Pigment Indexes	**EPI**	$Car = \alpha \times \left(R_{672}/\left(R_{550} \times R_{708}\right)\right)^{\beta}$	Datt (1998)

Table 3. Spectral indexes developed as anthocyanin indicators.

Index	Short	Formula	Source
Anthocyanin Reflectance Index	**ARI**	$ARI = R_{550}^{-1} - R_{700}^{-1}$	Gitelson et al. (2001)
Modified Anthocyanin Reflectance Index	**mARI**	$mARI = \left(R_{530-570}^{-1} - R_{690-710}^{-1} \right) \times R_{NIR}$	Gitelson et al. (2006)
Red:Green Ratio	**RGR**	$RGR = R_R / R_G$	Gamon & Surfus (1999), Sims & Gamon (2002)

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Figure 5. (left) Mean reflectance spectra of leaves from two species of California oaks the winter deciduous *Quercus lobata* (Valley Oak) and evergreen *Q. agrifolia* (Live Oak), measured in midsummer at Jasper Ridge Biological Preserve. (right) Mean pigment concentration and composition for these species. (reproduced from Ustin et al., 1993).

Figure 6. Optimal positions of spectral band λ_1 of the model $[R(\lambda_1)^{-1}-R(\lambda_2)^{-1}]\times R(\lambda_3)$ for retrieval of (A) total chlorophyll, (B) carotenoid, and (C) anthocyanin content from reflectance spectra (reproduced from Gitelson et al., 2006)

Figure 7. Predicted and measured total chlorophyll (left) and total carotenoid (right) pigments based on an inversion of the PROSPECT 5 model (reproduced from Feret et al., 2008) using leaves from four sources: (● LOPEX (Hosgood et al., 1994) □ CALMIT (Gitelson et al., 2002, 2003)◆ ANGERS (INRA, France) ○ HAWAI (Asner and Martin, 2008).

Figure 1

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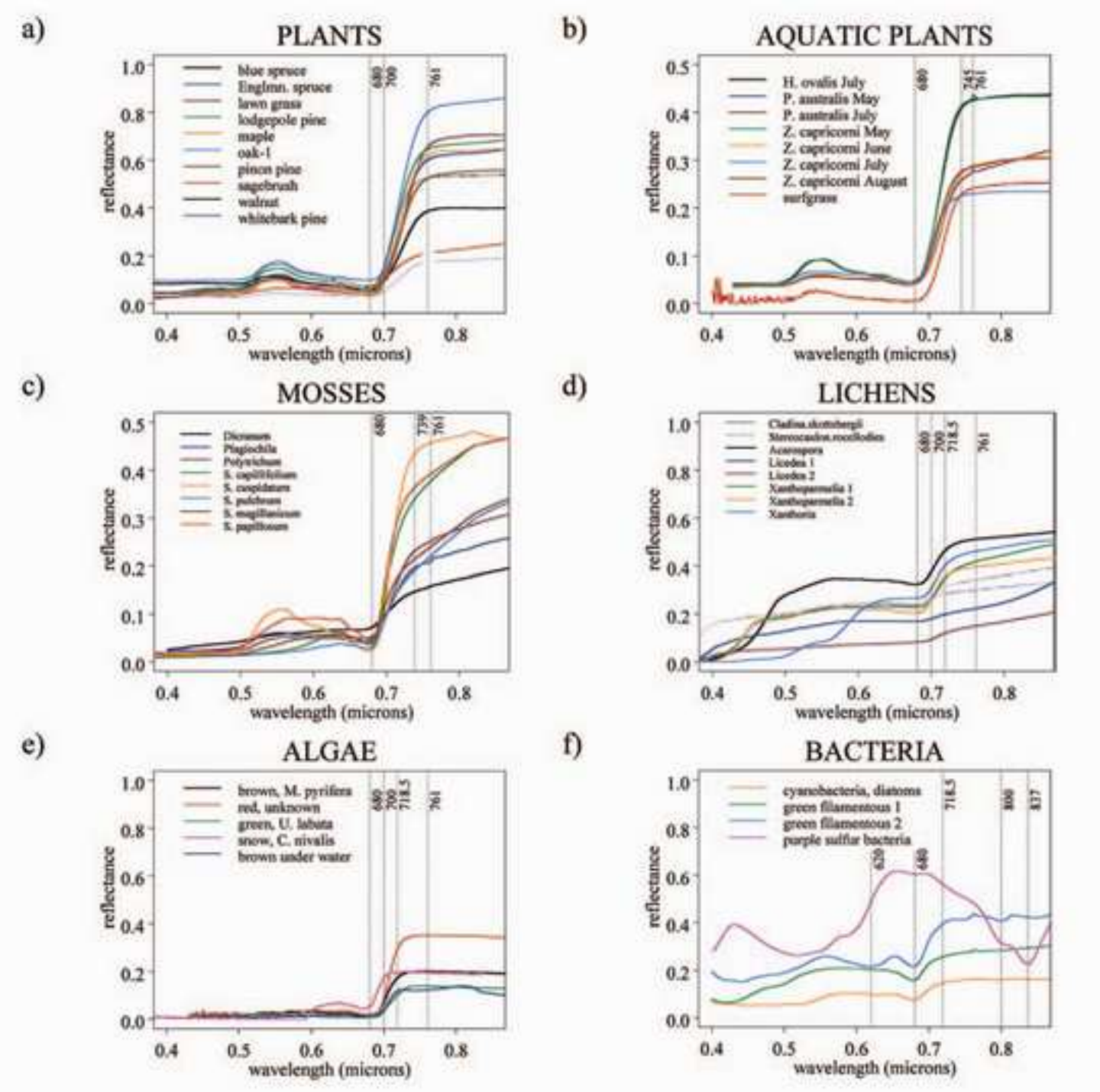

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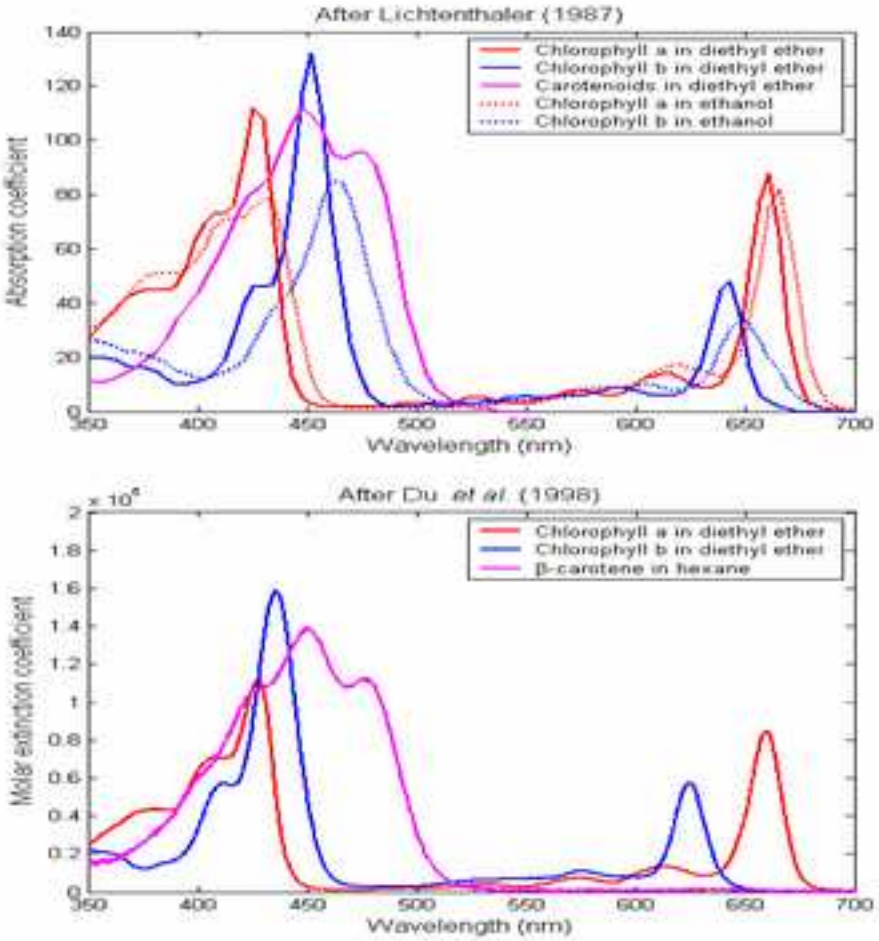

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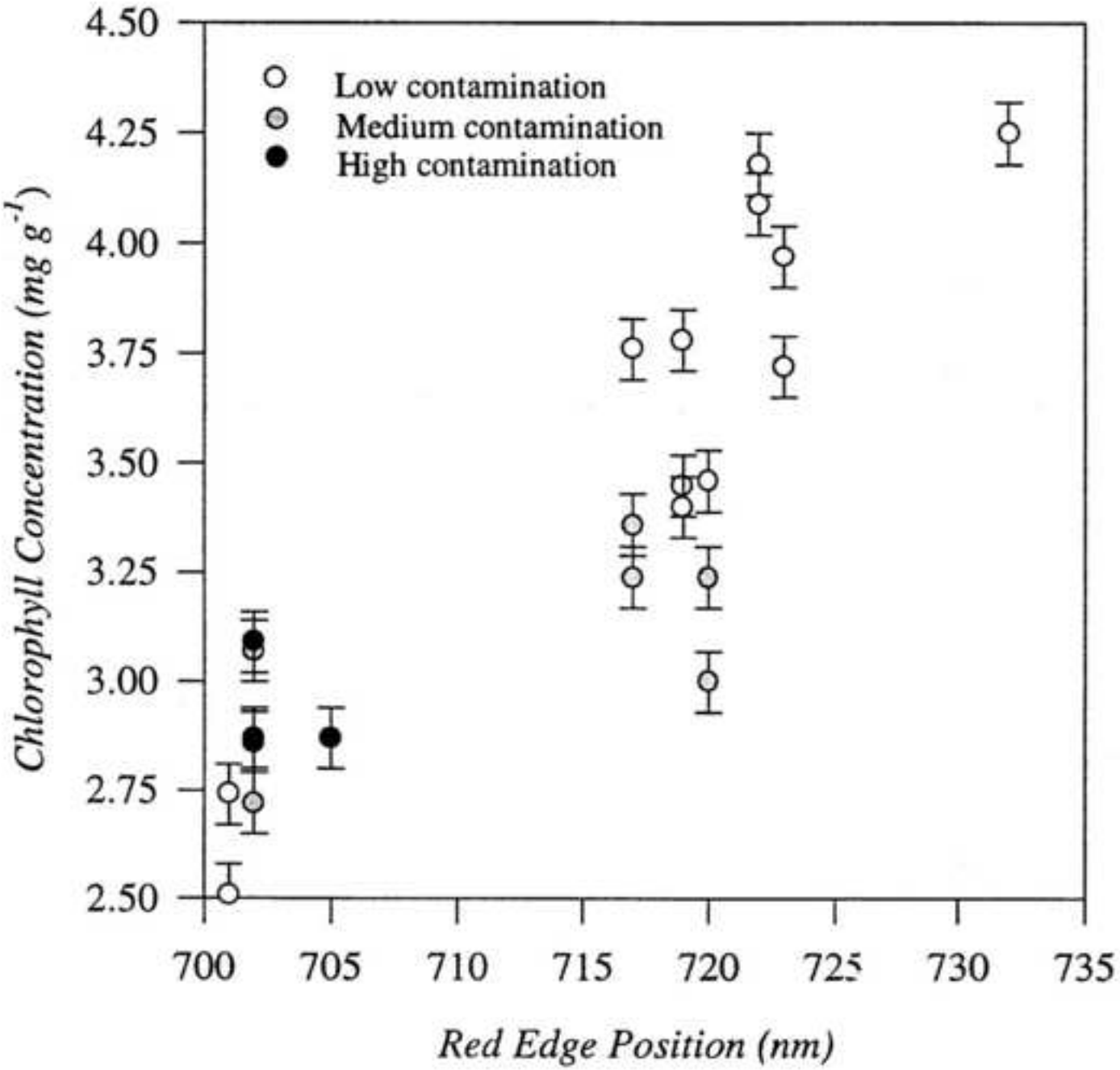

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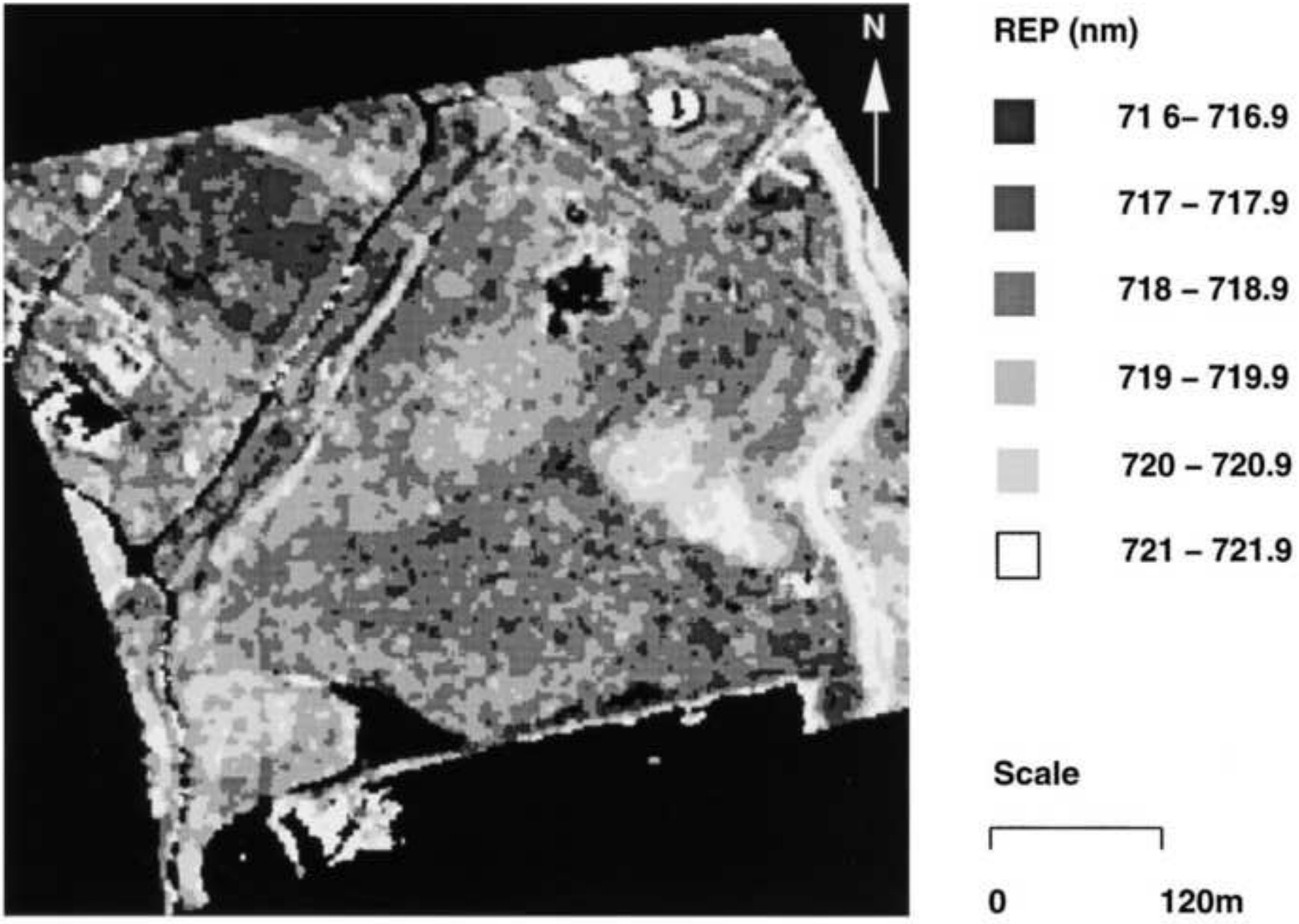

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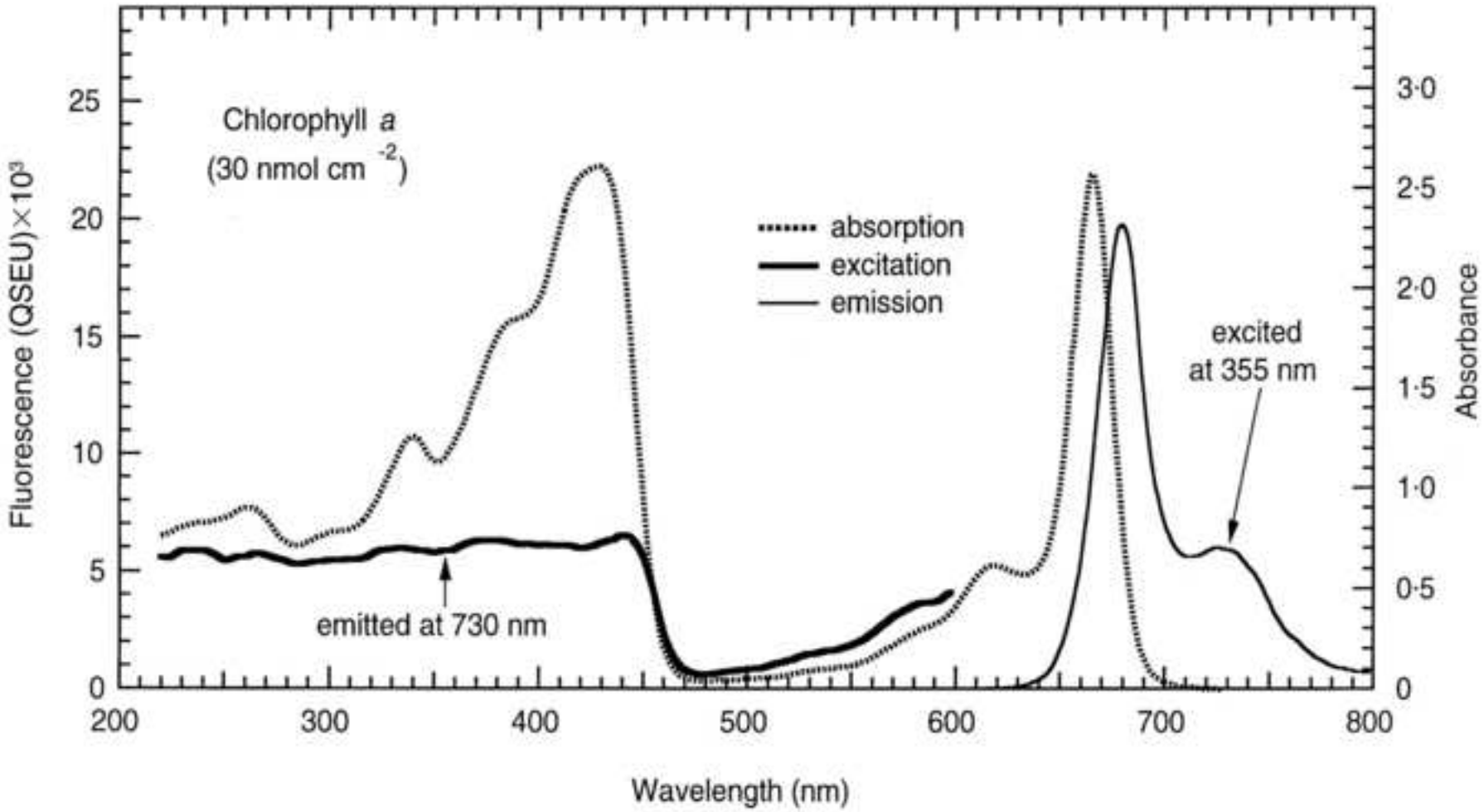

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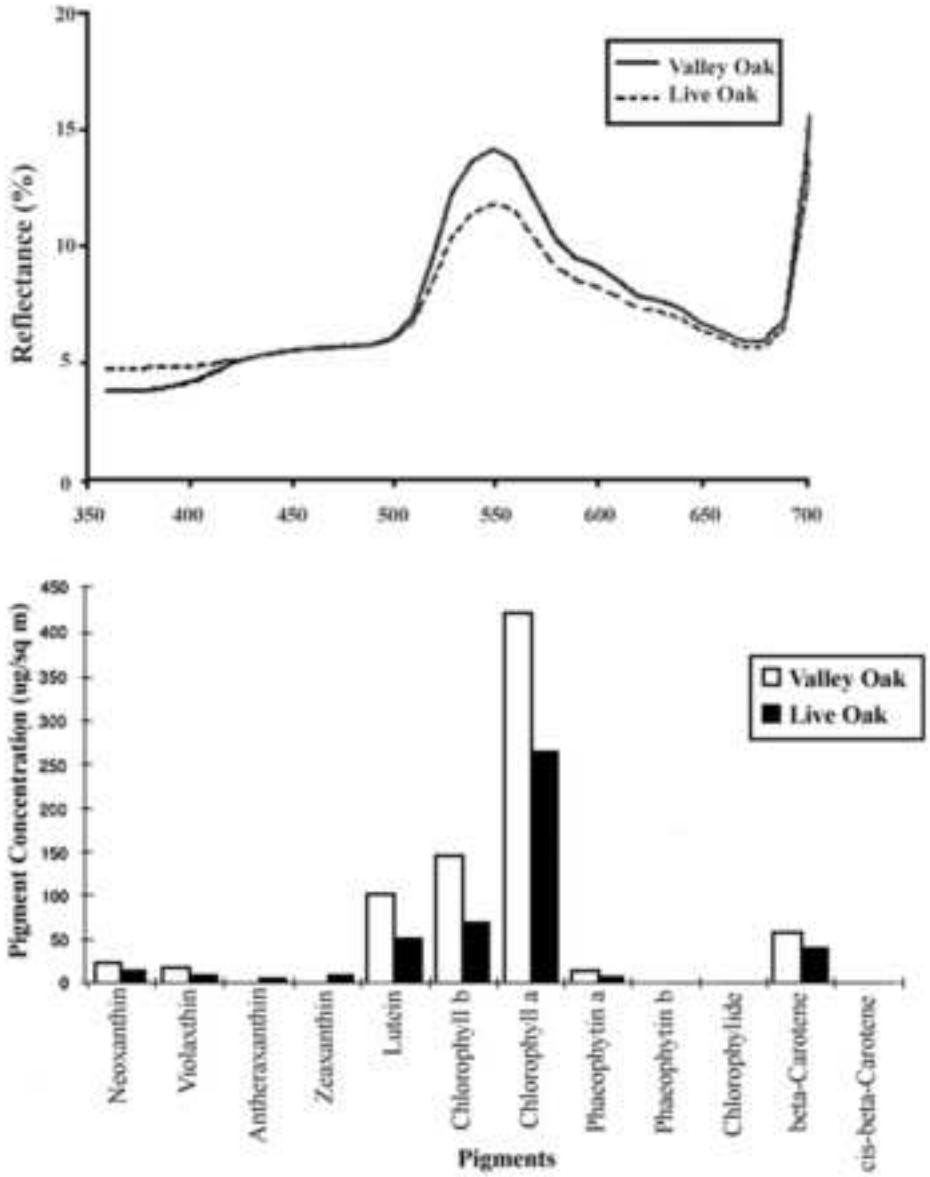

Figure 6

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